SHORT COMMUNICATION

Sexual receptivity varies according to female age in a Neotropical nuptial gift-giving spider

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Abstract. For many spiders, sex pheromones associated with female silk are important stimuli that elicit male searching and courtship behaviors. In that context, female sexual receptivity and chemical attractiveness can depend on age and reproductive status. In *Paratrechalea ornata* (Mello-Leitão 1943) (Araneae: Trechaleidae), males offer females a nuptial gift (a prey wrapped in silk) during courtship. Gift construction is elicited by the presence of female silk, and silk attractiveness is influenced by female age, increasing from 20 days after the female reaches adulthood. Our goal was to investigate whether female age affects female sexual receptivity and to discuss the relationship between receptivity and silk attractiveness. We exposed 26 virgin females, from 2 to 28 days after the final molt, to males offering a nuptial gift. Female sexual receptivity was age dependent and increased with adult female age. Females over 15 days from adulthood accepted more gifts than younger females, but the latency of female gift acceptance was not affected by female age. Female sexual receptivity is synchronized with chemical attractiveness, suggesting that females' pheromone release is adjusted at a particular mating age. We suggest that young virgin females may invest more in foraging and maturing gonads than in mating, accounting for the delay in receptivity and chemical attractiveness.

Keywords: Chemical attractiveness, age dependence, receptive females, *Paratrechalea ornata*

Reproduction occurring soon after sexual maturity can benefit individuals by increasing the number of offspring in a reproductive season and/or accelerating offspring reproduction (Moore & Moore 2001; Oli et al. 2002). However, these benefits may be balanced by associated costs such as reductions in growth, survival, or future reproduction (Stearns 1989). In fact, age can affect female fitness, and in some species it has been suggested that early mating is suboptimal for females (Krüger 2005; Maklakov et al. 2007). Consequently, young virgin females can sometimes delay first mating and reject males, for instance, when they are not physiologically mature (Markow 2000). However, resisting and rejecting behaviors may be costly for females due to male harassment (Rowe et al. 1994); females can decrease costs by being undetectable and avoiding direct contact with males (Wilcox 1984; Krupa et al. 1990). Sex pheromones, especially those presented in the silk (contact sex pheromones), are a very important communicatory channel associated with male attraction in spiders (Gaskett 2007). In some species, female sexual receptivity and chemical attractiveness depend on adult age and reproductive status (Roberts & Uetz 2005; Baruffaldi & Costa 2010). In this context, females may have the opportunity to maintain their crypticity when the costs of mating are high (Stoltz et al. 2007).

In the spider species *Paratrechalea ornata* (Mello-Leitão 1943) (Araneae: Trechaleidae), the male offers the female a prey wrapped in silk during courtship (Costa-Schmidt et al. 2008; Albo & Costa 2010). Since there is no report of gift stealing during courtship and before mating in this species, once the female accepts the nuptial gift by grasping it with her chelicerae, the male adopts the mating position and starts sperm transfer. Albo et al. (2009) reported age dependence in both male and female gift construction behaviors. Older males, exposed to female silk and/or female presence, are more prone to construct nuptial gifts than younger ones. Similarly, older females elicit more gift constructions than younger females, suggesting that female attractiveness is affected by female age.

If chemical attractiveness is linked to sexual receptivity, we predict younger females to be less sexually receptive (more reluctant) to male

courtship than older ones. Our aim in this study is to elucidate the relationship between adult age and sexual receptivity by exposing *P. ornata* females of different ages to courting males, monitoring the occurrence and latency of female gift acceptance. We discuss the results, taking into account a previous study of female chemical attractiveness in *P. ornata* (Albo et al. 2009).

Paratrechalea ornata is a South American crepuscular/nocturnal and semi-aquatic spider (Carico 2005). In Uruguay, this species has two reproductive seasons (April–July and September–December), apparently with no overlap (Albo & Costa unpublished data). We collected 52 subadults during September and October 2008 in Yerbal Chico Stream (Quebrada de los Cuervos, Department of Treinta y Tres, 32°55′30.50″S, 54°27′33.10″W), Uruguay. To accelerate maturation we housed spiders in individual glass jars (8 cm high × 11 cm diameter) in a warm room at an average temperature of 26.6°C (± 1.1 SD). We measured age from date of sexual maturity (day of final molt). Male age averaged 37.4 days (± 32.3 SD), with no interaction with gift acceptance or female age ($X^2 = 0.03$, $df = 1$, $P = 0.85$; $X^2_{M*F} = 2.36$, $df = 1$, $P = 0.12$). After the spiders molted, we maintained them and carried out the experiments in the same room at 23.1°C (± 2.2 SD). Individuals were fed weekly with a mixed diet of termites (*Nasutitermes* sp.), fruit flies (*Drosophila melanogaster*) and pieces of mealworm (*Tenebrio molitor*). Water was provided daily with moist cotton wool.

We carried out the experiments after sunset in large glass cages (30 cm long, 15 cm wide, 20 cm high), each containing a layer of pebbles and a Petri dish with water, from 17 September to 26 December 2008. We exposed 26 virgin females, ranging in age from 2–28 days (after final molts), to males offering a nuptial gift. Males and females were fed two fruit flies (*Drosophila melanogaster*) on the day before the experiment to standardize feeding conditions before the experiment; afterwards females were transferred to experimental glass cages. Each experiment consisted of three steps. First, we placed the male in a Petri dish containing haphazard female silk, offering a large fruit fly (*Drosophila funebris*) and eliciting prey-wrapping behavior (Albo et al. 2009). Second, seven minutes after the last silk wrapping, we transferred the male with the gift to the experimental glass cage, where he made contact with the female silk but remained separated from the female by
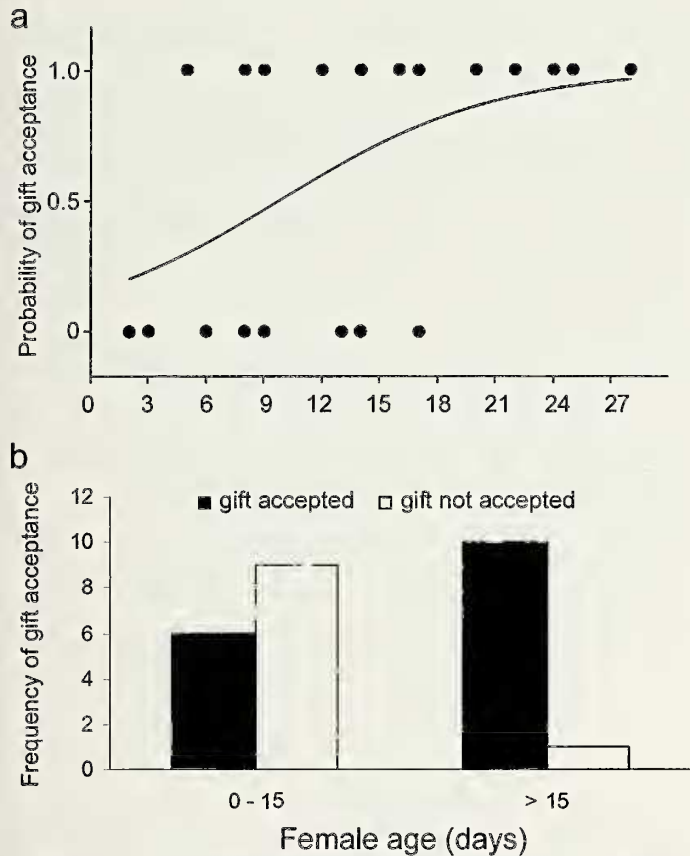
Figure 1.—Occurrence of female gift acceptance a) according to female age and b) before and after 15 days of adulthood.

Figure 2.—Latency of gift acceptance (in minutes) according to female age (in days).

an opaque wall. Third, after five minutes for male habituation to the new environment, we removed the wall allowing a male-female encounter. The experiment concluded when the female accepted the gift or after female rejected the male. We recorded female gift acceptance when the female grasped the gift offered by the male, and female rejection of the male when the female ran away without grasping the gift. We registered both frequency and latency of female gift acceptance. Latency of gift acceptanee was measured from the point of first encounter between the sexes to the point when the female grasped the gift with its chelicerae. Voucher males and females were deposited in the arachnological collection of the Facultad de Ciencias, Montevideo, Uruguay.

Data analysis was performed with PAST statistical package (Hammer et al. 2003). We tested normality of residuals and homogeneity of variances with Shapiro-Wilk and Levene tests, respectively. For comparing mean values, we used one-way ANOVA and the Student's t test for two independent samples. We used logistic regression to assess whether adult age affected gift acceptance and Fisher's exact probability test adjusted to multiple comparisons (Bonferroni correction) to compare frequencies of gift acceptance. The data were transformed whenever necessary to meet parametric assumptions.

We found that female gift acceptance varied according to female age. Female sexual receptivity increased correspondingly with the number of days after reaching adulthood ($X^2 = 7.45$, $P = 0.006$; Fig. 1a). When we analyzed the data in detail, we found that females more than 15 days past the final molt (older females) were more receptive and showed 90% gift acceptance, while females less than 15 days past the final molt (younger females) rejected males offering gifts more often (only 38% gift acceptance) (Fisher exact test, with Bonferroni correction: $P = 0.02$; Fig. 1b). Reluctant females rejected
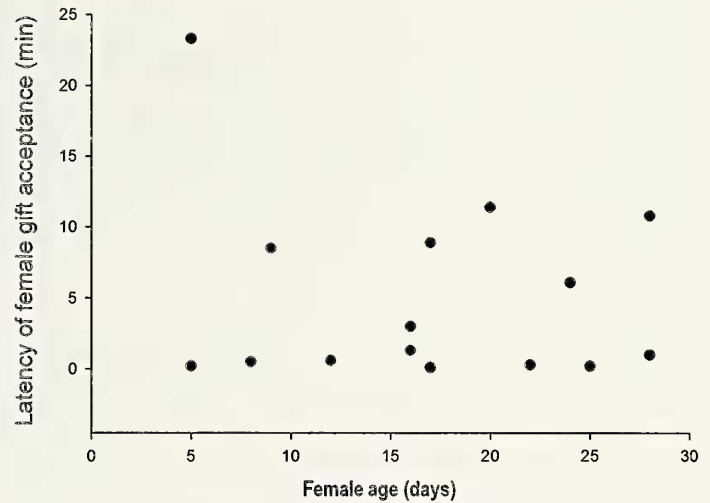
males by running away without grasping the gift, while receptive females stayed close to the courted male and grasped the gift.

We did not find statistical differences in the latency of gift acceptance either among different female ages ($F_{1,13} = 0.001$, $P = 0.99$; Fig. 2) or between the two female age categories, being 6.62 min ($\pm$ 9.9 SD) in younger females and 4.31 min ($\pm$ 4.5 SD) in older females (Student t-test: $t_{10.8} = 0.010$, $P = 0.99$). No qualitative differences were observed between female adult age and male courtship and/or gift offering behaviors.

Our findings show that female sexual receptivity and mate acceptance are influenced by female age, as was previously indicated in cockroaches and spiders (Moore & Moore 2001; Uetz & Norton 2007). Fifteen days after reaching adulthood, females accept mates more frequently, suggesting an optimal age for starting sexual activities. Adaptively and similar to young virgin females of many animal species, *P. ornata* females may be investing in other activities such as foraging and gonad maturation before starting investment in reproduction (Markow 2000; Bukowski & Avilés 2002; Krüger 2005). Maklakov et al. (2007) reported in a seed beetle that early matings are suboptimal for females, since females mating early in life suffer from a reduction in lifetime fecundity. Consequently, female attractiveness may be adaptively adjusted to minimize possible encounters with males early in their adult lives (Wilcox 1984; Krupa et al. 1990; Stoltz et al. 2007). For instance, Schulz & Toft (1993) and Schulz (2004) indicated that female spiders may control their attractiveness by varying their pheromone emissions. In the wolf spider *Schizocosa malitiosa* (Tullgren 1905), females reach their maximal chemical attractiveness 20 days after reaching adulthood (Baruffaldi & Costa 2010). Similarly, Albo et al. (2009) reported that the silk of mature *P. ornata* virgin females is more chemically attractive to males after 20 days of adulthood and elicits more frequent male gift construction than that of younger females.

Adult female *P. ornata* can live approximately 90 days in the field (Albo & Costa unpublished data). Hence, the difference in days between sexual receptivity (15 days after reaching sexual maturity) and chemical attractiveness (20 days after reaching sexual maturity) is only 6% of a female's adult lifespan. This suggests that pheromone release and female sexual attraction are adjusted to sexual maturity at a particular mating age. From the male perspective, finding young females, despite their low receptivity, may be beneficial. Because they are entelegyne spiders, being the first to mate with a female probably increases a male's percentage of paternity due to first male sperm priority (Austad 1984; but see Uhl 2000). In addition, females producing a suboptimally attractive pheromone could be indirectly

selecting good searcher males (Svensson 1996; Jaffe et al. 2007; Baruffaldi & Costa 2010), which may explain cases of female acceptance of males within 15 days of reaching maturity in this study.

In conclusion, it seems that female age, sexual receptivity, and chemical attractiveness are important factors influencing female reproductive decisions in *P. ornata*. We need further studies testing the effects of female age on reproductive success (i.e., copulation duration and number of fertilized eggs), allowing for the estimation of probable costs associated with early reproduction in this species.

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